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 NEWS  1           Web Page URLs for STN Seminar Schedule - N. America
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 NEWS  3  FEB 25   CA/CAPLUS - Russian Agency for Patents and Trademarks
                   (ROSPATENT) added to list of core patent offices covered
 NEWS  4  FEB 28   PATDPAFULL - New display fields provide for legal status
                   data from INPADOC
 NEWS  5  FEB 28   BABS - Current-awareness alerts (SDIs) available
 NEWS  6  FEB 28   MEDLINE/LMEDLINE reloaded
 NEWS  7  MAR 02   GBFULL: New full-text patent database on STN
 NEWS  8  MAR 03   REGISTRY/ZREGISTRY - Sequence annotations enhanced
 NEWS  9  MAR 03   MEDLINE file segment of TOXCENTER reloaded
 NEWS  10 MAR 22   KOREAPAT now updated monthly; patent information enhanced
 NEWS  11 MAR 22   Original IDE display format returns to REGISTRY/ZREGISTRY
 NEWS  12 MAR 22   PATDPASPC - New patent database available
 NEWS  13 MAR 22   REGISTRY/ZREGISTRY enhanced with experimental property tags
 NEWS  14 APR 04   EPFULL enhanced with additional patent information and new
                   fields
 NEWS  15 APR 04   EMBASE - Database reloaded and enhanced

 NEWS EXPRESS  JANUARY 10 CURRENT WINDOWS VERSION IS V7.01a, CURRENT
               MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 10 JANUARY 2005

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=> s hairpin RNA
L1 1217 HAIRPIN RNA

=> s l1 and mammalian cell
L2 41 L1 AND MAMMALIAN CELL

=> s l1 and mammalian cell#
4 FILES SEARCHED...
L3 224 L1 AND MAMMALIAN CELL#

=> s l3 and ((attenuat? or modulat?) (10a) expression)
L4 8 L3 AND ((ATTENUAT? OR MODULAT?) (10A) EXPRESSION)

=> dup rem l4
PROCESSING COMPLETED FOR L4
L5 8 DUP REM L4 (0 DUPLICATES REMOVED)

=> d ibib abs l5 1-8

L5 ANSWER 1 OF 8 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-21922 BIOTECHDS
TITLE: Selectively reducing expression of coding sequence in mammalian target cell, useful for determining target gene function, by introducing RNA interference agent specific for coding sequence into mammalian target cell; small interfering RNA or short **hairpin** **RNA** transfer and expression in host cell for gene therapy
AUTHOR: CHI J; CHANG H; WANG N; CHANG D; DUNPHY N; BROWN P
PATENT ASSIGNEE: UNIV LELAND STANFORD JUNIOR
PATENT INFO: WO 2004078950 16 Sep 2004
APPLICATION INFO: WO 2004-US7053 4 Mar 2004
PRIORITY INFO: US 2003-452379 5 Mar 2003; US 2003-452379 5 Mar 2003
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-662423 [64]
AN 2004-21922 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Selectively reducing (M1) expression of a coding sequence in a mammalian target cell, involves introducing into the mammalian target cell an RNA interference (RNAi) agent specific for the coding sequence to selectively reduce expression of the coding sequence.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a pharmaceutical preparation (I) comprising an RNAi agent in a delivery vehicle; and (2) a kit (II) for use in (M1), comprising (I) and instructions for carrying out (M1).

BIOTECHNOLOGY - Preferred Method: In (M1), the RNAi agent is an interfering RNA such as small interference (siRNA) or shRNA. The RNAi agent is a transcription template of an interfering RNA. The transcription template is a DNA, which encodes a shRNA. The **mammalian cell** is present in vitro or in vivo.
Preferred Pharmaceutical Preparation: (I) further comprises an RNAse inhibitor. Preferred Kit: (II) further comprises an RNAse inhibitor.

ACTIVITY - None given.

MECHANISM OF ACTION - **Modulator** of target gene

expression (claimed). No supporting data is given.

USE - (M1) is useful for selectively reducing expression of a coding sequence in a mammalian target cell (claimed). (M1) is useful for determining the function of a target gene or coding sequence in a **mammalian cell** and for determining potential targets for pharmaceuticals. (M1) is also useful in the treatment of a variety of different conditions in which the **modulation** of target gene **expression** in a mammalian host is desired.

ADMINISTRATION - (I) is administered by oral, buccal, rectal, parenteral, intraperitoneal, intradermal, or transdermal route. No specific dosage detail is given.

EXAMPLE - Specificity of small interference (siRNA) was evaluated using green fluorescent protein (GFP) of Aequoria victoria as a model target, as the protein level was easily monitored. The human embryonic kidney (HEK) 293 cells were transiently transfected with GFP and the two siRNAs directed toward GFP sequences (5'-CUACAACAGCCACAACGUCdTdTdTdTGAUGU UGUCGGUGUUGCAG-3', 5'-CAACAUCUCGACACCAGCAdTdTdTdTGUUGUAGAGCUGUGGUCGU-3'). Suppressed level of GFP activity by over 80%, was observed after transfection. Co-transfection of GFP with scrambled siRNAs matched for nucleotide content (termed C1 and C2) was unable to affect GFP activity compared to mock transfected cells, which were not exposed to siRNA. To test the specificity of RNAi against an integrated and nuclear gene, a population of cells stably expressing a GFP gene that was introduced by retroviral transduction was established. Transfection of the stable GFP-expressing cells with the above mentioned siRNAs silenced GFP expression by more than 70%, but GFP expression was unaffected by mock or C1 transfection. (33 pages)

L5 ANSWER 2 OF 8 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-16687 BIOTECHDS
TITLE: Making a transcription product of a target nucleic acid sequence, for diagnosing diseases in plants or animals, comprises admixing RNA polymerase, single-stranded transcription substrate and nucleoside triphosphates; involving Escherichia coli RNA-polymerase and polymerase chain reaction for use in disease diagnosis
AUTHOR: DAHL G A; JENDRISAK J J; DAVYDOVA E; ROTHMAN-DENES L; GERDES S
PATENT ASSIGNEE: EPICENTRE TECHNOLOGIES
PATENT INFO: WO 2004048594 10 Jun 2004
APPLICATION INFO: WO 2003-US37356 21 Nov 2003
PRIORITY INFO: US 2002-428013 21 Nov 2002; US 2002-428013 21 Nov 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-450394 [42]
AN 2004-16687 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Making a transcription product corresponding to a target nucleic acid sequence comprises admixing RNA polymerase, a single-stranded transcription substrate and nucleoside triphosphates (NTPs), and incubating the RNA polymerase and the single-stranded transcription substrate to allow synthesis of transcription product.

DETAILED DESCRIPTION - Making transcription product corresponding to a target nucleic acid sequence comprising: (a) obtaining an RNA polymerase that can transcribe RNA using a single-stranded promoter; (b) obtaining a single stranded DNA comprising a target nucleic sequence that is present in or complementary to at least a portion of a target nucleic acid in a sample; (c) obtaining a single-stranded transcription substrate by operably joining to the single-stranded DNA a single-stranded polynucleotide comprising a promoter sequence that binds the RNA polymerase; (d) obtaining NTPs that are substrates for the RNA polymerase and that are complementary to canonical nucleic acid bases; (e) admixing the RNA polymerase, single-stranded transcription substrate and NTPs; and

(f) incubating the RNA polymerase and the single-stranded transcription substrate to allow synthesis of transcription product. INDEPENDENT CLAIMS are also included for the following: (1) obtaining additional rounds of synthesis of transcription product corresponding to a target nucleic acid sequence; (2) **attenuating expression** of a target gene in a cell; (3) a **hairpin RNA** made by the method above; (4) a cell comprising the **hairpin RNA**; (5) a kit for performing the method above (or for making the hairpin above), the kit comprising an RNA polymerase defined above and a promoter splice template oligo, promoter ligation oligo or promoter primer (or an oligonucleotide comprising a sequence corresponding to a single-stranded promoter sequence); (6) cloning a target nucleic acid; (7) constructing a nucleic acid library comprising clones of substantially all nucleic acids or all mRNAs within a sample by using the method of (6); (8) a composition comprising a clone made by the method of (6) or a nucleic acid library made by using the method of (7); (9) a host cell comprising a circular DNA molecule made by using the method of (6); (10) a circular DNA molecule made by using the method of (6); (11) a kit for performing the method of (6); and (12) detecting an analyte in a sample.

BIOTECHNOLOGY - Preferred Method: Making transcription product may comprise: (a) obtaining an RNA polymerase that can transcribe RNA using a single-stranded promoter; (b) obtaining a single stranded DNA comprising a target nucleic sequence that is present in or complementary to at least a portion of a target nucleic acid in a sample; (c) obtaining a single-stranded transcription substrate by operably joining to the single-stranded DNA a single-stranded polynucleotide comprising a promoter sequence that binds the RNA polymerase; (d) admixing the RNA polymerase and the single-stranded transcription substrate; and (e) incubating the RNA polymerase and the single-stranded transcription substrate to allow synthesis of transcription product. The RNA polymerase comprises a region encoding a polypeptide having a sequence of 3500, 1107, 1143, 1143, or 3537 amino acids (SEQ ID NO: 2, 4, 6, 8, or 15, respectively) fully defined in the specification. It may comprise a polypeptide encoded by the nucleic acid sequence of 10506, 3318, 3432, 3432, or 10617 bp (SEQ ID NO: 1, 3, 5, 7, or 14, respectively) also given in the specification. The promoter is an N4 virion RNA polymerase (vRNAP) promoter comprising the sequence: ggcattacttcatccaaaagaagcggagcttc (SEQ ID NO: 16); ggcattacttcatccaaaagaagctgagcttc (SEQ ID NO: 19); caacgaagcgttgaatacct (SEQ ID NO: 27); ttcttcgaggcgaagaaaacct (SEQ ID NO: 28); or cgacgaggcgtcgaaaacca (SEQ ID NO: 29). The promoter may be a P2 sequence of SEQ ID NO: 16 or 28. The RNA polymerase is Escherichia coli RNAP and the promoter is a single-stranded pseudopromoter for E. coli RNAP. The RNA polymerase may be a T7-type RNAP and the promoter is a cognate single-stranded pseudopromoter for the T7-type RNAP. The RNA polymerase is a T7 RNAP and the promoter is a single-stranded pseudopromoter for T7 RNAP. The RNA polymerase is T3 RNAP or SP6 RNAP and the promoter is a single-stranded pseudopromoter for T3 RNAP or SP6 RNAP, respectively. The single-stranded DNA comprises a target nucleic acid sequence comprising an RNA or mRNA target nucleic acid or mRNA target nucleic acid that is full-length or corresponding to substantially all mRNA in the sample. The single-stranded DNA comprises a target nucleic acid sequence comprising a DNA target nucleic acid (that is a product of an amplification reaction). The amplification reaction is PCR, Reverse Transcription-PCR, Nucleic Acid Sequence Based Amplification, TMA, 3SR, Ligase Chain Reaction, Linked Linear Amplification, Strand Displacement Amplification, RCA, Multiple Displacement Amplification, Ican (RTM), Ucan (RTM), Loop-AMP (RTM), Spia (RTM) or Ribo-Spia (RTM). The single-stranded DNA comprises a target nucleic acid sequence that is obtained by primer extension of a larger DNA target nucleic acid or by reverse transcriptase primer extension of at least one mRNA target nucleic acid or of substantially all mRNA target nucleic acid in a sample. The single-stranded DNA comprises a target nucleic acid sequence that has a tail sequence comprising at least two nucleotides, dCMP nucleotides or a

tail sequence between 2-10 nucleotides. The single-stranded transcription substrate of step (c) is obtained using a promoter splice template oligo. The single-stranded DNA is obtained by reverse transcription of a transcription product. The single-stranded DNA in step (c) is obtained by reverse transcription of a transcription product prepared using the method above. The single-stranded DNA transcription substrate is obtained by using a promoter ligation oligo and a ligation splint. The single-stranded DNA may be obtained by reverse transcription of a transcription product prepared using a promoter ligation oligo and a ligation splint. The single-stranded transcription substrate may be obtained by DNA polymerase-catalyzed primer extension of a promoter primer using the target nucleic acid in the sample as a template, followed by ligation of the 5'-end of the primer-extended promoter primer to the 3'-end primer extension, comprising the single-stranded DNA comprising the target nucleic acid sequence, thus operably joining the promoter to the target nucleic acid sequence to form a circular single-stranded transcription substrate. The target nucleic acid in the sample comprises RNA and DNA polymerase used for primer extension is an enzyme with reverse transcriptase activity. The target nucleic acid in the sample may comprise mRNA. The method further comprises the DNA polymerase used for primer extension is an enzyme with reverse transcriptase activity. The single-stranded DNA comprising a target nucleic acid sequence is obtained by reverse transcription of a transcription product prepared using the method above. A linear single-stranded transcription substrate is obtained by cleaving a circular single-stranded transcription substrate obtained using the method above at a site that is 3' of the promoter sequence and 5' of the target nucleic acid sequence. The single-stranded DNA comprising a target nucleic acid sequence is obtained by reverse transcription of a transcription product prepared using the method above. At least one of the NTPs comprises a 2'-aminodeoxynucleoside triphosphate, 2'-amino-dCTP, 2'-fluorodeoxynucleoside triphosphate, 2'-fluoro-dCTP, 2'-fluoro-dUTP, 2'-azido-deoxynucleoside triphosphate, or 2'-azido-dCTP. The NTPs are complementary to canonical nucleic acid bases. The target nucleic acid sequence comprises a 3' portion that encodes a first sequence, a 5' portion that encodes a second sequence that is complementary to the first sequence, and a middle portion that joins the 3' portion and 5' portion, where the middle portion comprises a sequence that is not complementary to either the 3- portion or 5' portion and the transcription product comprises a **hairpin RNA**. The **hairpin RNA** corresponds to a target nucleic acid sequence in a target nucleic acid comprising an mRNA. The **hairpin RNA** may have an RNA interference activity in a cell that synthesizes as mRNA target nucleic acid comprising the target nucleic acid sequence. The **hairpin RNA** may comprise siRNA or at least one modified NTP defined above. The **hairpin RNA** is at least 40 or 100 nucleotides in length. The **hairpin RNA** is made with an RNA polymerase defined above that can use a single-stranded promoter for transcription of RNA. The **hairpin RNA** is made in vitro or in vivo. Obtaining additional rounds of synthesis of transcription product corresponding to a target nucleic acid sequence comprises: (a) obtaining an RNA polymerase that can transcribe RNA using a single-stranded promoter; (b) obtaining a first transcription product by transcription of a first single-stranded transcription substrate comprising a polynucleotide corresponding to a target nucleic acid sequence; (c) obtaining a reverse transcriptase; (d) reverse transcribing the first single-stranded transcription product; (e) obtaining first-strand cDNA complementary to the first single-stranded transcription product; (f) obtaining a second single-stranded transcription substrate by operably joining to the first-strand cDNA a single-stranded polynucleotide comprising a promoter sequence that binds the RNA polymerase; (g) admixing the RNA polymerase and the second single-stranded transcription substrate; and (h) incubating the RNA

polymerase and the second single-stranded transcription substrate to synthesize a second transcription product. Preferred Kit: The RNA polymerase comprises E. coli RNAP and the promoter splice template, ligation oligo, or promoter primer comprises a single-stranded pseudopromoter for E. coli RNAP. The RNA polymerase comprises a T7-type RNAP and the promoter splice template oligo, ligation oligo or promoter primer comprises a single-stranded pseudopromoter for the T7-type RNAP. The RNA polymerase comprises T7 RNAP and the promoter splice template oligo, ligation oligo or promoter primer comprises a single-stranded pseudopromoter for T7 RNAP. The RNA polymerase comprises T3 RNAP or SP6 RNAP and the promoter splice template oligo, ligation oligo or promoter primer comprises a single-stranded pseudopromoter for T3 RNAP or SP6 RNAP, respectively. **Attenuating expression** of a target gene in a cell comprises introducing the **hairpin RNA** defined above into the cell. The **expression** of a target gene in the cell is **attenuated** in vitro or in vivo. The cell comprises a **mammalian cell** which comprises a human cell. Cloning a target nucleic acid comprises: (a) obtaining a single-stranded DNA comprising a target nucleic acid sequence that is present in or complementary to the target nucleic acid; (b) joining to the single-stranded DNA a single-stranded polynucleotide comprising a single-stranded origin of replication and a marker gene; (c) making a circular single-stranded DNA molecule by covalently joining the 3'-end and 5'-end of the product of step (b); (d) transforming the circular single-stranded DNA molecule into a host cell, in which the marker gene is expressible, where the host cell is capable of replicating the circular single-stranded DNA molecule; and (e) growing the host cell under conditions that support the expression of the marker gene. The single-stranded polynucleotide comprises a single-stranded origin of replication and a marker gene are joined to the single-stranded DNA by using a promoter splice template oligo or promoter ligation oligo. The single-stranded origin of replication comprises an M13 origin of replication. The marker gene comprises an antibiotic-resistance gene. The marker gene comprises a beta-galactosidase gene. The single-stranded polynucleotide comprises a transposon recognition sequence or a site than can be recognized by a recombinase. The circular single-stranded DNA molecule is made by using a ligase that catalyzes non-homologous intramolecular ligation, where the ligase is ThermoPhage (RTM) RNA Ligase II. The circular DNA molecule is made by DNA polymerase-catalyzed primer extension of a primer using the target nucleic acid as a template, followed by ligation of the 3'-end of the primer extension product to the 5'-end of the primer extension product, where the primer comprises a single-stranded origin of replication and a marker gene. The single-stranded polynucleotide comprises a single-stranded promoter that binds a RNA polymerase defined above that can transcribe RNA using a promoter that is single-stranded. The single-stranded polynucleotide comprises a single-stranded promoter that binds a RNA polymerase that can transcribe RNA using a promoter that is single-stranded and where the host cell comprises an expressible gene encoding an RNA polymerase that can transcribe RNA using the single-stranded promoter. The expressible gene is operably joined to an inducible promoter that is a bad promoter, a lac promoter, a trp promoter, a tac promoter or a lambda promoter. Detecting an analyte in a sample comprises: (a) obtaining a transcription signaling system comprising a single-stranded DNA comprising a promoter sequence that binds an RNA polymerase that can transcribe RNA using a single-stranded promoter and a signal sequence that is operably joined to the promoter sequence; (b) joining the transcription signaling system to an analyte-binding substance; (c) contacting the analyte-binding substance to which the transcription signaling system is joined with a sample for binding of an analyte to the analyte-binding substance and forming a specific binding pair; (d) removing the specific binding pair from the sample; (e) incubating the specific-binding pair with an RNA polymerase defined above for the synthesis of a transcription product;

and (f) detecting the transcription product. The analyte is a biochemical molecule, a biopolymer, protein, glycoprotein, lipoprotein, enzyme, hormone, receptor, antigen, antibody, nucleic acid, DNA, RNA, polysaccharide or a lipid. The signal sequence comprises a substrate for Q-beta replicase or a sequence that encodes a detectable protein that is a green fluorescent protein. The signal sequence comprises a sequence that is detectable by a probe, preferably a sequence comprising a molecular beacon. The analyte-binding substance is nucleic acid, polynucleotide, oligonucleotide, a segment of a nucleic acid or polynucleotide, a DNA, RNA, a molecule comprising both DNA and RNA mononucleosides, modified DNA mononucleosides, a molecule obtained by a method termed SELEX, a nucleic acid molecule having an affinity for protein molecules, a polynucleotide molecule having an affinity for protein molecules, an operator, promoter, an origin of replication, restriction endonuclease recognition sequence, a ribosomal nucleic acid sequence, a sequence recognized by steroid hormone-receptor complexes, a peptide nucleic acid (PNA), nucleic acid and a PNA, a molecule prepared by using a combinatorial library of randomized peptide nucleic acids, an oligonucleotide or polynucleotide with a modified backbone that is not an amino acid, a molecule identified by using high throughput screening methods, lectin, a receptor for a hormone, a hormone, or an enzyme inhibitor. The binding of step (c) comprises non-covalent bonds comprising hydrogen-bonds, hydrophobic interactions, van der Waals forces or salt bridges.

USE - The method is useful for making transcription product (e.g., **hairpin RNA**) corresponding to a target nucleic acid sequence (claimed) to detect target nucleic acids in living cells. The method is useful for research, diagnostic and therapeutic applications, such as preparing cDNA corresponding to full-length mRNA, making sense or anti-sense probes, detecting gene- or organism-specific sequences, cloning, cell signaling, or making RNA for use in RNAi. The method is useful for diagnosing diseases in plants and animals, including humans, and for testing products such as food, blood and tissue cultures, for contaminants. The methods are useful for detecting cellular nucleic acids in whole cells from a specimen such as a fixed or paraffin-embedded section, or from microorganisms immobilized on a solid support such as replica-plated bacteria or yeast.

EXAMPLE - Each oligonucleotides (50 picomoles), comprising a sense P2 promoter sequence (or in control reactions, an anti-sense sequence to the P2 promoter or no promoter) at its 5'-end, which was phosphorylated, and up to 52 additional nucleotides corresponding to a model target sequence in its 3'-portion, was ligated in a reaction mixture for 2 hours at 60degreesC using 200 units of ThermoPhage (RTM) RNA Ligase II in 1X ThermoPhage (RTM) RNA Ligase II Buffer. Linear oligos were removed by digestion with Exonuclease I, the Exo I was heat-inactivated, and the circular single-stranded DNA (ssDNA) oligos where ethanol precipitated using standard techniques. One picomole of circular ssDNA oligonucleotide, was incubated for 4 hours at 37degreesC in reaction mixture comprising mini-virion RNA polymerase (vRNAP), nucleoside triphosphate, DTT and Escherichia coli SSB Protein in 1X Transcription Buffer. The resulting mini vRNAP transcription products were analyzed by electrophoresis. Transcription products, including those having a length many-fold greater than the starting oligonucleotide were observed on the gel, indicating efficient rolling circle transcription. No transcription products were observed in the control. (264 pages)

L5 ANSWER 3 OF 8 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-15332 BIOTECHDS
TITLE: Regulating a target gene at both transcription and post-transcription levels comprises introducing into a cell a zinc finger protein that can bind to a target gene or a nucleic acid encoding the protein, and a RNA molecule; antisense oligonucleotide and plasmid, virus or

liposome-mediated protein gene transfer to mammal cell for vascular endothelial cell growth factor-A gene regulation

AUTHOR: KIM J; SHIN H C; KWON H
PATENT ASSIGNEE: TOOLGEN INC
PATENT INFO: WO 2004044202 27 May 2004
APPLICATION INFO: WO 2003-KR2451 14 Nov 2003
PRIORITY INFO: KR 2002-70845 14 Nov 2002; KR 2002-70845 14 Nov 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-411728 [38]
AN 2004-15332 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Regulating a target gene comprising introducing into a cell a zinc finger protein that can bind to a target gene or a nucleic acid encoding the protein, and a RNA molecule comprising a strand that includes a sequence complementary to an mRNA transcribed from the target gene to regulate the expression of the target gene, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a composition for regulating a target gene, the composition comprising a zinc finger protein that can bind to the target gene or a nucleic acid encoding the protein; and a RNA molecule that includes a strand including a sequence complementary to an mRNA transcribed from the target gene; (2) treating a neoplastic disorder in a subject comprising providing the composition, and administering the composition to the subject to **modulate** VEGF-A gene **expression** in a cell of the subject; (3) a kit that comprises the zinc finger protein, and the RNA molecule; (4) a modified eukaryotic cell that comprises the zinc finger protein, and the RNA molecule; and (5) **modulating expression** of a target gene in a cell of a subject by administering a first component that comprises a zinc finger protein or a nucleic acid encoding the zinc finger protein, and a second component that comprises a RNA molecule or a nucleic acid that can produce the RNA molecule, to the subject, where the zinc finger protein can bind to the target gene and modulate transcription of the target gene, the RNA molecule can reduce translatability of a transcript of the target gene, and the first and second components are administered in amounts effective to **modulate expression** of the target gene in a cell of the subject.

BIOTECHNOLOGY - Preferred Method: In regulating a target gene, the zinc finger protein contains 3 to 6 zinc finger domains. The zinc finger domains are a wild-type, and/or non-wild-type. The zinc finger protein comprises a transcriptional repressor domain. The nucleic acid encoding the zinc finger protein is a DNA in the form of a plasmid. The zinc finger protein can bind to a regulatory sequence of the target gene. The zinc finger protein can bind to a promoter sequence, an enhancer sequence, a coding sequence, or an intronic sequence of the target gene. The promoter sequence of the target gene is a native one or an artificially substituted exogenous one. The promoter sequence of the target gene is modified to contain a recognition site for a zinc finger protein. The promoter sequence of the target gene is selected from VEGF promoter, TATA promoter, SV40 promoter and CMV promoter. The zinc finger protein binds to the sequence of the target gene with a KD of less than 10-7 M. The zinc finger protein alters expression of fewer than 5% of genes in the genome. The RNA molecule is selected from anti-sense RNA, ribozyme and double-stranded RNA. The RNA molecule is a double-stranded RNA, and each strand of the molecule has a size of 21 to 23 nucleotides. The double-stranded RNA has a double-stranded region of 19 nucleotides and 3'-end single-stranded regions of 2 to 4 nucleotides. The double-stranded RNA comprises two complementary RNA strands. The double-stranded RNA is a **hairpin RNA**. The RNA molecule is a chemically synthesized or naturally occurring derivative of RNA. The cell is a eukaryotic cell, preferably a **mammalian cell**. The cell is in an organism. The nucleic acid encoding the

zinc finger protein is introduced using a liposome, a virus, or a viral particle. The RNA molecule is introduced into the cell by inserting a nucleic acid that includes a sequence of a strand of the RNA molecule, or its complement into the cell, and transcribing or amplifying the nucleic acid in the cell, thus producing the RNA molecule in the cell. Preferred Composition: The composition comprises the double-stranded RNA comprising two complementary RNA strands. The double-stranded RNA is a **hairpin RNA**. The RNA molecule is a chemically synthesized or a naturally occurring derivative of RNA. The composition includes a zinc finger protein that comprises a protein transduction domain. The target gene is VEGF-A gene and the composition is used for **modulating** VEGF-A gene **expression**. Preferred Kit: The kit further comprises instructions for administering the kit components to a subject. The components are in separate containers. Preferred Cell: The modified eukaryotic cell comprises the target gene that is an endogenous gene of the cell. The RNA molecule is a double- stranded RNA molecule that comprises a duplex of less than 24 basepairs. The cell comprises a heterologous DNA molecule that produces the RNA molecule. In **modulating expression** of a target gene in a cell of a subject, the first and second components are administered separately or together. The RNA molecule is a double-stranded RNA molecule that comprises a duplex of less than 24 basepairs. The first and second components are formulated for delivery by a cell-penetrating vehicle. The cell-penetrating vehicle is a liposome, virus, or viral-like particle. The target gene promotes cell growth or proliferation. The target gene promotes inflammation. **Modulating expression** of a target gene comprises identifying a zinc finger protein that can bind to the target gene and modulate transcription of the target gene, identifying a RNA molecule can reduce translatability of a transcript of the target gene, the molecule comprising a strand that includes a sequence complementary to the transcript; and providing, in a form suitable for introduction into a cell or administration to a subject, a first component that comprises the identified zinc finger protein or a nucleic acid encoding the zinc finger protein, and a second component that comprises the identified RNA molecule or a nucleic acid that can produce the RNA molecule into a cell. The first and second components are combined and formulated as a pharmaceutical composition. The first and second components are combined by preparing a nucleic acid vector that comprises one sequence corresponding to the first component and another sequence corresponding to the second component. Identifying the zinc finger protein comprises mixing and matching characterized zinc finger domains, and screening a library of zinc finger protein for a protein that alters the phenotype of a cell.

USE - The method is useful for regulating a target gene (claimed) at both transcription and post-transcription levels. (60 pages)

L5 ANSWER 4 OF 8 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2005-04438 BIOTECHDS
TITLE: Novel isolated thioaptamer mediating gene silencing, useful for mediating gene silencing of target gene in cell or organism, and for examining function of gene in cell or organism;
RNA thioaptamer isolation and sense and antisense sequence for use in gene silencing
AUTHOR: GORENSTEIN D G; YANG X; KANG J
PATENT ASSIGNEE: UNIV TEXAS SYSTEM
PATENT INFO: US 2004242521 2 Dec 2004
APPLICATION INFO: US 2004-758488 15 Jan 2004
PRIORITY INFO: US 2004-758488 15 Jan 2004; US 1999-425798 25 Oct 1999
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2005-063517 [07]
AN 2005-04438 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - An isolated thioaptamer (I) mediating gene silencing, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) producing (M1) a mature thioaptamer of 21-23 nucleotides in length, involves: (a) combining a double-stranded precursor thioaptamer with a soluble extract that mediates gene silencing, thus producing a precursor-extract mixture; and (b) maintaining the precursor-extract mixture under conditions in which the double-stranded thioaptamer is processed to the mature thioaptamer of 21-23 nucleotides in length; (2) a mature thioaptamer of 21-23 nucleotides produced by (M1); (3) a knockdown cell or organism (II) generated by the method of mediating gene silencing of a target gene in cell or organism by using (I); (4) a pharmaceutical composition (PC) comprising (I) having 21-25 nucleotides that mediates thioaptamer gene silencing, and an appropriate carrier; (5) a combinatorial thioaptamer library (L1) comprising (a) two or more unique thioaptamers that comprise a combination of backbone modifications and sequence that mediates gene silencing of an mRNA to which it corresponds, or (b) a bead library of thioaptamer libraries, where each bead comprises a thioaptamer library of imperfect complementarity to a target sequence for gene silencing; and (6) a one-bead, one-thioaptamer combinatorial library, comprising (a) two or more beads, where attached to each bead is unique thioaptamer comprising a single unique sequence, each of the unique thioaptamer comprises a unique mix of modified and unmodified nucleotides, and the thioaptamer mediates gene silencing of an mRNA to which it corresponds, or (b) two or more beads, where attached to each bead is a unique thioaptamer comprising an imperfect complementarity match to a target gene to form a thioaptamer bead, each unique thioaptamer-bead comprises a unique mix of modified and unmodified nucleotides, and the thioaptamer mediates gene silencing of an mRNA to which it has imperfect complementarity.

BIOTECHNOLOGY - Preferred Thioaptamer: (I) further comprises a terminal 3' hydroxyl group. (I) comprises ribonucleotides or deoxyribonucleotides. (I) comprises one or more of the following: rATP(alphaS), rUTP(alphaS), rGTP(alphaS), rCTP(alphaS), rATP(alphaS2), rUTP(alphaS2), rGTP(alphaS2) or rCTP(alphaS2). (I) comprise 21-25 nucleotides. (I) comprises a double-stranded thioaptamer with a perfect complementarity match to a target gene and gene silencing occurs by mRNA cleavage; an imperfect complementarity match to a target gene and gene silencing occurs by repressed translation of mRNA to protein; or a single-stranded thioaptamer with perfect complementarity match to a target gene and gene silencing occurs by mRNA cleavage. (I) comprises a portion of a RNA-induced silencing complex (RISC). (I) is produced by a DICER complex. (I) comprises a short interfering RNA (siRNA), micro, interfering RNA (miRNA), small, temporal RNA (stRNA), or a short, **hairpin RNA** (shRNA). (I) is further defined as a thioaptamer precursor that comprises a long dsRNA, or a 70 nucleotide stem-loop RNA (shRNA). (I) comprises a double-stranded thioaptamer of 21-25 nucleotides long, or a single-stranded thioaptamer that is 15-22 nucleotides long. The gene silencing is defined further as degradation of an mRNA transcript that is cleaved in the presence of (I) before it can express a protein, or as regulation of translation when (I) binds an mRNA transcript at or its 3'UTR. Preferred Method: (M1) further involves isolating (I) having 21-23 nucleotides from the precursor-extract mixture, determining the sequence of the mature thioaptamer and the location of one or more thio-modifications to the mature thioaptamer, and chemically synthesizing the mature thioaptamer. Preferred Knockdown Cell: (II) mimics a disease, where the cell is a stem cell. Preferred Library: In L1, the thioaptamers are attached covalently to one or more beads, where the beads are polystyrene/polydivinyl benzene copolymer. (I) comprise one or more phosphorothioate, phosphorodithioate or methylphosphonate linkages. (I) comprises a viral protein sequence, genomic sequence or expressed sequence, and further comprise a colorimetric agent. L1 further comprises the complementary strand to (I).

(I) is created by a split and pool combinatorial synthesis chemistry.

ACTIVITY - Cytostatic. No supporting data is given.

MECHANISM OF ACTION - Antisense therapy.

USE - (I) is useful for mediating gene silencing of a target gene in a cell or organism, which involves introducing (I) having 21-23 nucleotides, into the cell or organism, and maintaining the cell or organism under conditions in which gene silencing occurs, thus mediating inhibition of the expression of the target gene in the cell or organism. (I) is optimized for RNase H degradation of the message. The target gene encodes viral gene or cellular gene. The gene silencing is defined further as degradation of an mRNA transcript of the target gene that is cleaved in the presence of (I) before it can express a protein, or as regulation of translation of the target gene when (I) binds an mRNA transcript of the target gene at or its 3'UTR. (I) is useful for examining the function of a gene in a cell or organism, which involves introducing (I) having 21-23 nucleotides that targets an mRNA of the gene for gene silencing, into the cell or organism, thus producing a test cell or test organism; maintaining the test cell or test organism under conditions under which gene silencing of mRNA of the gene occurs, thus producing a test cell or test organism in which in mRNA of the gene is silenced; and observing the phenotype of the test cell or test organism against an appropriate control cell or control organism to provide information about the function of the gene. (I) is useful for assessing whether a gene product is a suitable target for drug discovery, which involves introducing (I) such as an RNA thioaptamer that mediates gene silencing of 21-25 nucleotides, into a cell or organism under conditions in which gene silencing of an mRNA for the target gene results in decreased expression of the gene, and determining the effect of the decreased expression of the gene on the cell or organism, where if decreased expression has an effect, then the gene product is a target for drug discovery. (I) is useful for identifying target sites within an mRNA that are efficiently targeted for gene silencing, which involves: (a) combining (I) such as an RNA thioaptamer corresponding to a sequence of a labeled mRNA to be degraded under conditions in which labeled mRNA is degraded; or (b) combining (I) such as an RNA thioaptamer corresponding to a sequence of a labeled mRNA under conditions in which labeled mRNA is not degraded and the protein level is reduced. The method further involves the step of identifying one or more sites in the mRNA that are efficiently cleaved. The RNA thioaptamer is defined further as a thioaptamer library, or as a pool of thioaptamer from a thioaptamer library. (I) is useful for reducing the expression of a gene in a cell, which involves selecting (I) that mediates gene silencing of the gene to which it corresponds, and introducing (I) into the cell, where (I) mediates RNA interference of a targeted sequence. The targeted sequence is chosen from markers, splice acceptors, splice donors, internal ribosome entry sites (IRES), recombinase sites, promoters, ori sequences, cloning sites and intervening sequence. The targeted sequence comprises viral, autologous or heterologous sequence. The cell is a **mammalian cell**, preferably the stem cell of the human.

(I) is an antisense molecule, ribozyme or double-stranded RNA (dsRNA). The gene is associated with a disease or disorder. (I) is useful for **attenuating expression** of a target gene in cultured cells, which involves: (a) introducing (I) such as RNA thioaptamer, into the cells in an amount sufficient to **attenuate expression** of the target gene, where the RNA thioaptamer comprises a nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of the target gene and mediates **attenuation** of protein **expression** for a gene to which it corresponds; or (b) introducing (I) into the cell, in an amount sufficient to **attenuate expression** of the target gene, where (I) mediates gene silencing of a nucleic acid to which it hybridizes under stringent conditions, and activating a gene silencing activity in the cell. The cell is in cell culture, or is a cell infected

with a virus (all claimed).

ADVANTAGE - (I) such as RNA thioaptamers, enables gene silencing, including degradative and non-degradative interference with translation. (I) enables rapid detection, isolation and evaluation of siRNA oligonucleotides that have reduce susceptibility to nucleases, that are sequence specific, have a gene silencing activity that is equal to the wild-type siRNA. (I) specifically binds to a target molecule or its portion and mediate gene silencing of target gene without manifesting side effects on other genes of the cell, and also enables detection of gene silencing. (40 pages)

L5 ANSWER 5 OF 8 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-15286 BIOTECHDS
TITLE: **Attenuating expression** of target gene of cell in vivo useful for treating e.g. myocardial infarction and cancer, involves administering RNAi constructs e.g. small interfering RNA formulated in supramolecular complex or liposome;
target gene **expression attenuation** and RNA interference for use in gene therapy
AUTHOR: DAVIS M E; JENSEN G S; PUN S H
PATENT ASSIGNEE: DAVIS M E; JENSEN G S; PUN S H
PATENT INFO: US 2004063654 1 Apr 2004
APPLICATION INFO: US 2003-440506 15 May 2003
PRIORITY INFO: US 2003-440506 15 May 2003; US 2001-336314 2 Nov 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-346270 [32]
AN 2004-15286 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - **Attenuating** (M1) **expression** of a target gene of a cell in vivo or of patient, involves administering RNAi constructs (I), formulated in a supramolecular complex or liposomes in an amount sufficient to **attenuate expression** of the target gene through an RNA interference mechanisms, and thus alter the growth, survival or differentiation of treated cells.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a stable respiratory formulation (II) comprising (I) formulated for pulmonary or nasal delivery of (I) to the lungs of a patient; (2) a metered dose aerosol dispenser (III) containing an aerosol composition for pulmonary or nasal delivery comprising (II); (3) a preparation (IV) comprising (II) and a carrier; (4) a composition (V) comprising: (a) one or more (I) formulated in a supramolecular complex and in an amount sufficient to **attenuate expression** of a target gene in treated cells through an RNA interference mechanism; (b) one or more (I) formulated for percutaneous intrapericardial delivery to an animal; (c) one or more (I) formulated in liposomes for **attenuating expression** of a target gene of cells in vivo through an RNA interference mechanism; (d) one or more (I) formulated for electroporation into cells in vivo; and (e) one or more formulated (I) for inhibiting unwanted cell growth in vivo, where through an RNA interference mechanism, (I) reduces expression of a target gene essential to mitosis of a cell and/or which is essential to preventing apoptosis of the cell; (5) a preparation (IVa) comprising (Va), (Vb), (Vc), (Vd), or (Ve) and a carrier; (6) a package (VI) comprising (IV) or (IVa), in association with instructions (written and/or pictorial) for administering the preparation to a human patient; (7) a coating (VII) for use on a surface of a medical device, comprising a polymer matrix having (I) dispersed in it, where (I) are eluted from the matrix when implanted at site in a patient's body and alter the growth, survival or differentiation of cells in the vicinity of the implemented device; (8) coating (M2) a medical device with one or more (I), involves formulating (I) for coating a surface of a device such that (I) are eluted from the

surface when the device is implanted at site in a patient's body, and coating the formulated (I) on a medical device, where the medical device coated with (I) **attenuates expression** of one or more genes in cells in the vicinity of the implanted device; and (9) a cosmetic preparation (VIII) comprising (Ve), where (I) inhibits epithelial cell growth or differentiation. Further INDEPENDENT CLAIMS are detailed in Technology Focus Field.

BIOTECHNOLOGY - Preferred Formulation (claimed): a polymeric formulation (XI) for double stranded RNA, comprising a double-stranded RNA admixed with a polymeric agent in an amount that the resulting polymeric formulation has one or more of the following characteristic relative to the double stranded RNA alone such as a longer half-life in serum, an increased therapeutic index and/or a decreased ability to stimulate an immune response. Preferred Methods (claimed): (1) conducting (M6) a pharmaceutical business, involving: (a) identifying (I) which inhibits proliferation of target cells in vivo and reduces the effects of a disorder involving unwanted proliferation of the target cells; (b) conducting therapeutic profiling of (I) identified in the above step for efficacy and toxicity in animals; and (c) formulating a preparation including one or more (I) identified as having an acceptable therapeutic profile, or involves performing step (a), optionally step (b), and licensing, to a third party, the rights for further development of (I). (2) inducing (M5) cell death, involves administering to a target cells in vivo an double stranded RNA, or an expression vector capable of transcribing a double stranded RNA, of sufficient length to activate a PKR response in the target cells, which double stranded RNA is formulated as part of a supramolecular complex; (3) delivering (M4) one or more (I) to a patient by electroporation, involves administering (I) of sufficient amount to an animal through electroporation, where (I) **attenuates expression** of a target gene in cells of the patient; (4) percutaneous intrapericardial delivery (M3) of one or more (I) in vivo, involves administering a formulation of (I) to the pericardial space of an animal, where (I) are present in an amount sufficient to **attenuate expression** of one or more target genes of cells of the treated animal; Preferred Method: In (M1), (I) is an small-interfering RNA (siRNA) which is 19-30 base pairs long. The siRNA is single strand of siRNA. (I) is an expression vector having a coding sequence that is transcribed to produce one or more transcriptional products that produce siRNA in the treated cells. (I) is a **hairpin RNA** which is processed to siRNA in the treated cells. The supramolecular complex is a multi-dimensional polymer network including linear polymers or branched polymers. The supramolecular complex is formed from cationic polymers chosen from poly(L)lysine (PLL), polyethylenimine (PEI), beta-cyclodextrin containing polymers (betaCD-polymers), and their co-polymers. The supramolecular complex is formed cyclodextrin-modified polymers. The supramolecular complex is formed from cyclodextrin-modified poly(ethylenimine) and has a structure of formula (1). R = H, lower alkyl, cyclodextrin moiety or formula (2); and m = 2-10000, preferably 10-5000 or 100-1000. The supramolecular complexes are aggregated into particles having an average diameter of 0.5 and 200 microns. The particles have an average diameter of 0.5-10 microns. In (M2), (I) **attenuates expression** of a gene resulting in reduced proliferation and/or migration of smooth muscle cells. In (M3), the pericardial space is used as a delivery reservoir for (I). (I) is delivered locally to the heart and surrounding vasculature. In (M4), (I) is formulated in supramolecular complexes or liposomes. In (M5), the double stranded RNA is more than 35 bp in length, preferably more than 75 nucleotides. The target cells are **mammalian cells** or transformed cells. The supramolecular complex is a multi-dimensional polymer network including linear polymers or branched polymers. (M6) further involves establishing a distribution system for distributing the preparation for sale, and (optionally) establishing a sales group for marketing the preparation. Preferred Formulation: In

(II), (I) are formulated as microparticles having average diameter less than 20 microns, preferably 0.5-10 microns. The microparticles are formed from biodegradable polymers. The microparticles are formed from one or more polymers chosen from polysaccharides, diketopiperazines, poly(hydroxy acids), polyanhydrides, polyesters, polyamides, polycarbonates, polyalkylenes, poly vinyl compounds, polysiloxanes, polymers of acrylic and methacrylic acids, polyurethanes, celluloses, poly(butic acid), poly(valeric acid), and poly(lactide-co-caprolactone), or their co-polymers. The microparticles are formed by solvent evaporation, spray drying, solvent extraction or hot melt encapsulation. The microparticles are in dry or lyophilized form. (I) are formulated as supramolecular complexes including a multi-dimensional polymer network. (I) are formulated in liposomes. (II) includes a propellant. (II) is contained in a metered dose inhaler, a dry powder inhaler or an air-jet nebulizer. (I) is formulated in an amount to provide one to ten meter doses. (I) includes modifications to either phosphate-sugar backbone or the nucleoside. (I) includes a backbone modification chosen from phosphorothioates, phosphoramidate, phosphodithioates, chimeric methylphosphonate-phosphodiesters, peptide nucleic acids, and 5-propynyl-pyrimidine containing oligomers. (IX) is a supramolecular complex. The polymeric agent is a cationic polymer or cyclodextrin-modified polymer. The double stranded RNA is an small-interfering RNA (siRNA). The double stranded RNA is a **hairpin RNA** which is processed to an siRNA in a treated cell. The double-stranded RNA is a long double stranded RNA which induces a PKR-dependent response in treated cells. (IX) effective when administered intravenously under conditions of normal venous pressure. Preferred Preparation: In (IV) or (IVa), the carrier is chosen from salts, ester, and salts of such esters. Preferred Composition: (Va) is aggregated into particles having an average diameter of 20-500 nm, preferably 20-200 nm. In (Vb), (I) is encapsulated or associated with liposomes. The liposomes are cationic formed from cationic vesicle-forming lipids. The liposomes have an average diameter of less than 200 nm. (Vd) is formulated for electroporation into cells such as epithelial cells or muscle cells. In (Ve), (I) is an expression vector chosen from episomal expression vector, integrative expression vector, or viral expression vector. The target gene is an oncogene chosen from c-myc, c-myb, mdm2, PKA-I, Abl-1, Bcl2, Ras, c-Raf kinase, CDC25 phosphatases, cyclins, cyclin dependent kinases, telomerases, PDGF/sis, erb-B, fos, jun, mos, src, and Bcr/Abl fusion gene. The cells is transformed cell. (I) is formulated in a supramolecular complex comprising at least one polymer which is a cyclodextrin containing polymer. The liposome complex with (I) has a substantially homogeneous size of typically less than 200 nm. Preferred Coating: In (VII), the medical device is chosen from screw, plate, washers, suture, prosthesis anchor, tack, staple, electrical lead, valve, membrane, catheter, implantable vascular access port, blood storage bag, blood tubing, central venous catheter, arterial catheter, vascular graft, intraaortic balloon pump, heart valve, cardio vascular suture, artificial heart, pacemaker, ventricular assist pump, extracorporeal device, blood filter, hemodialysis unit, hemoperfusion unit, plasmapheresis unit, and filter adapted for deployment in a blood vessel, or medical device is a stent. In (VII), (I) attenuates at least one target gene chosen from cyclin dependent kinases, c-myb, c-myc, proliferating cell nuclear antigen (PCNA), transforming growth factor-beta (TGF-beta), and transcription factors nuclear factor kappaB (NF-kappaB), E2F, HER-2/neu, PKA, TGF-alpha, EGFR, TGF-beta, IGFIR, P12, MDM2, BRCA, Bcl-2, VEGF, MDR, ferritin, transferrin receptor, IRE, C-fos, HSP27, C-raf and metallothionein genes.

ACTIVITY - Cardiant; Cytostatic; Antiinflammatory; Vasotropic; Hypotensive; Antiatherosclerotic; Antirheumatic; Antiarthritic; Neuroprotective; Antidiabetic. No biological data given.

MECHANISM OF ACTION - Gene therapy; Inhibitor of **expression**

of gene to **attenuate** proliferation and/or migration of smooth muscle cells; **Attenuator** of **expression** of gene resulting in increased angiogenesis and/or reduced ischemic damage; Inhibitor of unwanted cell growth in vivo; Inhibitor of attenuator of hyperplastic cell growth; Inhibitor of activation of lymphocytes; Inhibitor of proliferation of epithelial cells; Inhibitor of expression of gene essential for proliferation of transformed cell such as tumor cell; Inhibitor of expression of gene which encode or regulate expression of cytokines; Inhibitor of expression of gene implicated in the onset or progression of diabetes; Inhibitor of expression of intracellular adhesion molecule (ICAM-1).

USE - (M1) is useful for **attenuating expression** of a target gene of a cell in vivo or of a patient. (M1) is for treatment of cells in vivo or in vitro. (M1) is useful for inhibiting unwanted cell growth in vivo which involves administering to an animal a formulated (I) of sufficient amount, where through an RNA interference mechanism, (I) reduces expression of a target gene essential to mitosis of a cell and/or which is essential to preventing apoptosis of the cell. (II) is useful for affecting systemic administration of (I) which involves administering (II) to a patient, by way of pulmonary administration, which is taken up in an amount in the deep lung to deliver a systemic dose of (I). (Vb) is useful for **attenuating expression** of a gene resulting in increased angiogenesis and/or reduced ischemic damage in and around a myocardial infarct. (I) is systemically available and **attenuates expression** of one or more genes in cells distal to the pericardial space. (Vb) is delivered to human. (Vc) is useful for **attenuating expression** of target gene of cells such as **mammalian cell** (human cell). (Ve) is useful for inhibiting unwanted cell growth in vivo. (I) of (Ve) inhibits proliferation of the cell or promotes apoptosis of the cell. (I) of (Ve) is used for the treatment of hyperplastic cell growth, such as cancer, inhibiting activation of lymphocytes for treatment or prophylaxis of immune mediated inflammatory disorders, inhibiting proliferation of smooth muscle cells for treatment or prophylaxis of restenosis, or inhibiting proliferation of epithelial cells, for cosmetic preparation. By (M3), (I) is used for reducing proliferation and/or migration of smooth muscle cells and for treating myocardial infarction (claimed). (M1) is useful for treating myocardial infarction, preventing apoptosis of cell, and cancer. (M1) is useful for treatment of prophylaxis of immune mediated inflammatory disorders and restenosis. (M1) is useful for inhibiting proliferation of epithelial cells and thus (I) is useful as a component of cosmetic preparations. (M1) is useful for treating neointimal hyperplasia such as restenosis, atherosclerosis, etc. (M1) is useful for treatment or prophylaxis of neoplastic, anaplastic and/or hyperplastic cell growth, tumor, for anti-cancer treatment, and chronic lymphatic leukemia. (M1) is useful for treatment or prophylaxis of rheumatoid arthritis, inflammation and inflammation related diseases such as multiple sclerosis and diabetes. (M1) is used in treating psoriasis, acute renal failure, reperfusion injury and prolonging renal isograft survival. (V) is useful for reducing expression of vasoconstrictors or reducing receptor levels of vasoconstrictor, reducing blood pressure in patients suffering from systemic and pulmonary hypertension.

ADMINISTRATION - (I) is administered by pulmonary or nasal, percutaneous intrapericardial, or intravenous route. No dosage detail is given. (39 pages)

L5 ANSWER 6 OF 8 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

ACCESSION NUMBER: 2004:935836 SCISEARCH
THE GENUINE ARTICLE: 862WF
TITLE: Influence of casein kinase II in tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis in human rhabdomyosarcoma cells

AUTHOR: Izeradjene K; Douglas L; Delaney A; Houghton J A (Reprint)
CORPORATE SOURCE: St Jude Childrens Hosp, Dept Hematol Oncol, Div Mol Therapeut, 332 N Lauderdale St, Memphis, TN 38105 USA (Reprint); St Jude Childrens Hosp, Dept Hematol Oncol, Div Mol Therapeut, Memphis, TN 38105 USA
COUNTRY OF AUTHOR: USA
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LANGUAGE: English
REFERENCE COUNT: 58

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) induces apoptosis via the death receptors DR4 and DR5 in transformed cells in vitro and exhibits potent antitumor activity in vivo with minor side effects. Protein kinase casein kinase II (CK2) is increased in response to diverse growth stimuli and is aberrantly elevated in a variety of human cancers. Rhabdomyosarcoma tumors are the most common soft-tissue sarcoma in childhood. In this investigation, we demonstrate that CK2 is a key survival factor that protects tumor cells from TRAIL-induced apoptosis. We have demonstrated that inhibition of CK2 phosphorylation events by 5,6-dichlorobenzimidazole (DRB) resulted in dramatic sensitization of tumor cells to TRAIL-induced apoptosis. CK2 inhibition also induced rapid cleavage of caspase-8, -9, and -3, as well as the caspase substrate poly(ADP-ribose) polymerase after TRAIL treatment. Overexpression of Bcl-2 protected cells from TRAIL-induced apoptosis in the presence of the CK2 inhibitor. Death signaling by TRAIL in these cells was Fas-associated death domain and caspase dependent because dominant negative Fas-associated death domain or the cowpox interleukin 1beta-converting enzyme inhibitor protein cytokine response modifier A prevented apoptosis in the presence of DRB. Analysis of death-inducing signaling complex (DISC) formation demonstrated that inhibition of CK2 by DRB increased the level of recruitment of procaspase-8 to the DISC and enhanced caspase-8-mediated cleavage of Bid, thereby increasing the release of the proapoptotic factors cytochrome c, HtrA2/Omi, Smac/DIABLO, and apoptosis inducing factor (AIF) from the mitochondria, with subsequent degradation of X-linked inhibitor of apoptosis protein (XIAP). To further interfere with CK2 function, JR1 and Rh30 cells were transfected with either short **hairpin RNA** targeted to CK2alpha or kinase-inactive CK2alpha (K68M) or CK2alpha' (K69M). Data show that the CK2 kinase activity was abrogated and that TRAIL sensitivity in both cell lines was increased. Silencing of CK2alpha expression with short **hairpin RNA** was also associated with degradation of XIAP. These findings suggest that CK2 regulates TRAIL signaling in rhabdomyosarcoma by **modulating** TRAIL-induced DISC formation and XIAP **expression**.

L5 ANSWER 7 OF 8 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-23041 BIOTECHDS
TITLE: **Attenuating expression** of a target gene in host cells comprises introducing double stranded RNA into the host cells in an amount that **attenuates expression** of the target gene;
ds RNA and small interfering RNA transfer and expression in stem cell for RNA interference, transplantation and gene therapy
AUTHOR: BEACH D H; BERNSTEIN E; CAUDY A; HAMMOND S; HANNON G J; PADDISON P J; CONKLIN D
PATENT ASSIGNEE: GENETICA INC; COLD SPRING HARBOR LAB
PATENT INFO: WO 2003062394 31 Jul 2003

APPLICATION INFO: WO 2003-US1963 22 Jan 2003
PRIORITY INFO: US 2002-55797 22 Jan 2002; US 2002-55797 22 Jan 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-636734 [60]
AN 2003-23041 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - **Attenuating** (M1) **expression** of a target gene in host cells comprising introducing double stranded RNA (dsRNA) into the host cells in an amount sufficient to **attenuate expression** of the target gene, where the dsRNA comprises a nucleotide sequence that hybridizes under stringent conditions to an untranslated or intronic sequence of the target gene, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a composition for **attenuating expression** of a target gene, comprising the above dsRNA or an expression vector which, when expressed, produces the dsRNA; (2) a pharmaceutical package comprising a pharmaceutical preparation of the dsRNA that is, or gives rise to, a short interfering dsRNA (siRNA) in a **mammalian cell** and **attenuates expression** of a target gene, which dsRNA does not produce a significant PKR-dependent response in the **mammalian cell** at concentrations effective for **attenuating expression** of the target gene, and label or instructions (written and/or pictorial) for administering the preparation to a patient; (3) generating siRNA; (4) performing a business of distributing siRNA; (5) altering the Major Histocompatibility Complex (MHC) phenotype of a donor stem cell or its progeny; (6) a culture of donor stem cells or their progeny; (7) performing a transplant on a patient; (8) reducing the susceptibility of host cells to infection by a pathogen; (9) a non-human transgenic mammal having germ line and/or somatic cells comprising a transgene encoding a dsRNA transcriptional produce that is processed to siRNA species, where transcription of the transgene **attenuates expression** of an endogenous target gene in at least one cell-type of the animal; and (10) a **hairpin RNA** comprising a first nucleotide sequence that hybridizes under stringent conditions to a nucleotide sequence of a target gene, and a second nucleotide sequence which is a complementary inverted repeat of the first nucleotide sequence and hybridizes to the first nucleotide sequence to form a hairpin structure, which **hairpin RNA attenuates expression** of the target gene and does not produce a significant PKR-dependent response at concentrations effective for **attenuating expression** of the target gene.

WIDER DISCLOSURE - Disclosed are: (a) an assay for identifying nucleic acid sequences responsible for conferring a particular phenotype in a cell; (b) conducting a drug discovery business; and (c) inhibiting RNAi or for altering the specificity of an RNA.

BIOTECHNOLOGY - Preferred Method: In **attenuating expression** of a target gene in host cells, the untranslated sequence of the target gene to which the dsRNA hybridizes is selected from a promoter sequence and an enhancer sequence. The host cells are suspended in culture and are in a whole animal. The target gene is a genomically integrated gene of the host cells. The target gene, which is a viral gene, is a heterologous gene. The host cell is a primate cell or a human cell. The dsRNA is siRNA that does not produce a significant PKR-dependent response in the host cells at concentrations effective for **attenuating expression** of the target gene. The dsRNA is 15-45 (preferably 19-30) bp in length. It is produced in the cell by an expression vector having a coding sequence that is transcribed to produce one or more transcriptional products that are processed to a siRNA species by the host cells. The dsRNA is a **hairpin RNA** that is processed to a siRNA species by the host cells. The **expression** of the target gene is **attenuated** by at least

10-fold. The siRNA hybridizes to an untranscribed sequence or a non-coding sequence of the target gene. Alternatively, the above method comprises administering to the mammal the composition cited above. **Attenuating** the **expression** of the target gene reduces unwanted growth or differentiation of the cells. Generating siRNA comprises: (a) providing an in vitro transcription system including a double stranded nucleic acid having complementary sense and antisense target sequences, which target sequence is flanked by promoters for an RNA polymerase; and the RNA polymerase, where the sense and antisense target sequences are transcribed and can anneal to form a siRNA; and (b) isolating the siRNA from the in vitro transcription system. The RNA polymerase is a bacteriophage RNA polymerase. It is selected from T3 polymerase, T7 polymerase and SP6 polymerase. The in vitro transcription system includes a variegated library of target sequences to produce a variegated library of siRNA species. Performing a business of distributing siRNA comprises accepting orders for siRNA species having a sequence designated by user, providing an in vitro transcription system mentioned above, isolating the siRNA from the in vitro transcription system, and packaging and shipping the siRNA to the user. Altering the MHC phenotype of a donor stem cell or its progeny comprises introducing the dsRNA into a stem cell in an amount that **attenuates expression** of an MHC gene otherwise expressed by the stem cell or its progeny. The dsRNA reduces the expression of one or more human leukocyte antigens (HLA) otherwise expressed by the stem cell or its progeny. Transplanting a patient comprises generating an ex vivo cell or tissue culture of donor stem cells, or their progeny, having an altered MHC phenotype resulting from stable **attenuation** of **expression** of one or more MHC genes by introduction of dsRNA into the donor stem cells, and transplanting the patient with the cell or tissue culture. Reducing the susceptibility of host cells to infection by a pathogen comprises introducing the dsRNA into the host cells in an amount that **attenuates** the **expression** of one or more genes necessary for **expression** by the pathogen. The pathogen is a virus and the dsRNA **attenuates expression** of cellular surface protein necessary for infection of the host cell by the virus. Preferred Transgenic Mammal: The transgenic mammal is chimeric for the transgene. The transgene is chromosomally incorporated and it transcribes separate complementary transcripts that anneal to form the siRNA. The transgene transcribes a **hairpin RNA** that is processed to the siRNA. Preferred RNA: The **hairpin RNA** is chemically synthesized or is enzymatically synthesized in vitro or in vivo. It is synthesized by T7 RNA polymerase in vitro and by RNA polymerase III in vivo. The **hairpin RNA** is produced by a vector. It has a size of about 20-50, 50-100 or 100-500 nucleotides. It includes a restriction enzyme site in the loop of the hairpin.

USE - M1 is useful in **attenuating** gene **expression** in a cell using gene-targeted dsRNA. The stem cell is useful in manufacturing a cellular medicament for transplantation to a patient, which cellular medicament comprises stem cells or their progeny which have an altered MHC phenotype resulting from stable **attenuation** of **expression** of one or more MHC genes by introduction of dsRNA into the stem cells. The composition is used in manufacturing a medicament for **attenuating expression** of one or more genes in vivo (all claimed).

EXAMPLE - No relevant example given. (150 pages)

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ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Small interfering RNA (siRNA) is a revolutionary tool for the experimental **modulation** of gene **expression**, in many cases making redundant the need for specific gene mutations and allowing examination of the effect of modulating essential genes. It has now been shown that siRNA phenotypes resulting from stable transfection with short **hairpin RNA** (shRNA) can be transmitted through the mouse germ line and Rosenquist and his colleagues have used shRNA, which is processed in vivo to siRNA, to create germline transgenic mice in which a target DNA repair gene has been silenced. Here, Holly Miller and Arthur R Grollman give the background of these discoveries, provide an overview of current uses, and took at future applications of this research.